

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.709.409>

Screening of *Pseudomonas fluorescens* against Dry Root Rot Pathogen *Macrophomina phaseolina* in Black Gram

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ABSTRACT

Black gram is one of the important pulse crops in India. Root rot of black gram caused by *Macrophomina phaseolina* is widely distributed in many countries and it is a devastating pathogen right from the establishment of the crop. Screening of *Psuedomonas fluorescens* was done against dry root rot pathogen *M. phaseolina* in black gram. The antagonistic activity of *P. fluorescens* against *M. phaseolina* was tested by dual culture technique. In the present study ten isolates of *P. fluorescens* were isolated on King's B medium from rizhosphere soil of different crops grown at the Agricultural College and Research Institute, Killikulam. The bacteria were identified as *P. fluorescens* Pfkkm1 - Pfkkm10. Among the ten isolates, seven isolates reduced the mycelial growth of *M. phaseolina*. Pfkkm7 recorded lowest mycelial growth of 10 mm with 88.5% reduction over control. This isolates also recorded max inhibition zone of 51mm. Pfkkm9 recorded mycelia growth of 43mm with 50.91% reduction over control and inhibition zone of 21.33mm. The other isolates like Pfkkm3, Pfkkm4 and Pfkkm10 reduced the mycelial growth but not to greater extent than Pfkkm7 and Pfkkm9. The isolates Pfkkm1, Pfkkm2, Pfkkm5, Pfkkm6, did not reduced the mycelia growth of *M. phaseolina*. Carbendazim reduced the dry root rot incidence and recorded lesser disease incidence of 18%. Among the two *P. fluorescens* isolates PFKKM7 recorded lesser disease incidence of 24%. *P. fluorescens* isolated Pfkkm9 also reduced the root rot incidence and recorded 40%. The inoculated control recorded the maximum disease incidence of 98%. The soil application of *P. fluorescens* isolates Pfkkm7 at the rate of 10g/kg of soil effectively reduced the dry root of black gram (75.5%).

Keywords

Black gram,
Macrophomina
phaseolina, *Psuedomonas*
fluorescens, Carbendazim

Article Info

Accepted:

24 August 2018

Available Online:

10 September 2018

Introduction

Black gram *Vigna radiata* (L.) constitutes the important group of grain legumes which form

a major source of dietary proteins of high biological value, energy, minerals and vitamins. The root rot disease caused by the soil borne fungus *Macrophomina phaseolina*

(Tassi) is a major limiting factor in the mung bean production causing considerable losses. The fungus *M. phaseolina* infects more than 500 plant species worldwide (Wyllie 1993) and causes charcoal rot disease in several agronomically important crops including soybean, maize, sorghum and cotton. Root rot of black gram caused by *M. phaseolina* is of considerable importance causing a loss up to 2.2-15.7%.

Management effective fungicide has been the most common practice till date. The alarming rate of environmental hazards both to flora and fauna, the increasing knowledge of too the effects on human health and an inclination towards sustainable agriculture has led to a more eco-friendly approach commonly known as.

Among the PGPR, fluorescent pseudomonads are the most preferred bacteria for biological control of soil-borne and foliar plant pathogens. On the above background an attempt was made to manage the root rot pathogen *M. phaseolina* using *P. fluorescence* (Saravanakumar *et al.*, 2007).

Materials and Methods

Isolation of *Macrophomona phaseolina* from infected black gram plants

Stem bark tissues of black gram bearing fungal sclerotia and characteristics root rot symptoms were collected for isolation of the pathogen. The tissues were cut into small pieces of 5-10 mm length and 2-3 mm thickness, surface sterilized with 1% mercuric chloride for 2 minutes and then rinsed thrice in sterile distilled water.

These pieces were placed on PDA Medium. The Petri dishes containing infected tissue were incubated in dark at $26 \pm 2^\circ\text{C}$ for 6 days. (Meyer *et al.*, 1973)

Isolation of *Pseudomonas fluorescens* from Rhizosphere soil

One gram of rhizosphere soil adhering to root surface was collected and transferred to a 250 ml conical flask containing 100 ml of sterile water. After thorough shaking for 15 minutes in a shaker, different dilutions were prepared. One ml of each 10^{-5} and 10^{-6} dilution was pipette out and poured into the sterile petridishes. Later King's medium B (KB) (Kings *et al.*, 1954) was poured, rotated and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 24 hours. After 24 hours of incubation, the bacterial growth was purified by the dilution plate technique (Wakes man and Connick, 1952). The bacterial culture was maintained in King's B broth (KB) in 30 percent (v/v) glycerol at -80°C .

In vitro* screening of *Pseudomonas fluorescens* against *Macrophomona phaseolina

The antagonistic activity of *P. fluorescens* against *Macrophomona phaseolina* was tested by dual culture technique (Dennis and Webster, 1971). Bacterial isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA. 9 mm mycelial disc from seven days old PDA culture of *Macrophomona phaseolina* was placed at the opposite side of petri dishes perpendicular to the bacterial streak and incubated at $28 \pm 2^\circ\text{C}$ for 5-7 days. Petri dishes inoculated with fungal discs alone served as control.

Three replications were maintained for each isolate. Observation on width of inhibition zone and mycelia growth of test pathogen was recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent (1927).

Per cent inhibition (I) = $\frac{C-T}{C} \times 100$;

Where: C- mycelial growth of pathogen in control and

T- Mycelial growth of pathogen in dual plate.

Development of talc -based formulation of Plant Growth Promoting Rhizobacteria

Ten gram of carboxy methylcellulose was mixed with 1 kg of talc powder and the pH was adjusted to 7.0 by adding 15 g of calcium carbonate. The mixture was then autoclaved for 30 minutes for two consecutive days. The *P.fluorescens* strains Pfkkm 7 and Pfkkm 9 were grown in King's B medium (KMB) and nutrient broth for 48 hours, respectively. 400 ml of the bacterial inoculum was then added to 1 kg of the talc mixture and mixed well under sterile conditions. The product was dried under shade to bring the moisture content to less than 20 per cent. The formulation was packed in polythene bags, sealed and kept under room temperature (Vidhyasekaran and Muthamilan, 1995).

Effect of *P. fluorescens* against dry root rot disease of black gram under pot culture experiment

Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 h for two consecutive days and filled in pots. The talc-based formulations of Pfkkm 7 and Pfkkm 9 were applied to the potting mixture at the rate of 10g per kg of soil. Carbendazim (fungicide) was used as a standard treatment for comparison. The fungicide was applied as soil drenching at the recommended dosage (1g/ liter of water). The culture of *Macrophomina phaseolina*, mass multiplied in sand maize medium (Riker and Riker, 1936) (sand and maize powder at the ratio of 19: 1) was incorporated with potting medium at the rate of 20g per kg of soil. The black gram variety Vamban 4 was sown at the rate of 10 seeds per pot and root rot disease incidence

was recorded on 45 days after sowing and expressed as percentage.

Statistical analysis

The results of all the experiments were analyzed independently. The treatment means were compared by Duncan's Multiple Range Test-DMRT (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1 developed by the International Rice Research Institute Biometrics unit, the Philippines.

Results and Discussion

Isolation of *Macrophomina phaseolina* from infected from black gram plants

The black gram plants showing typical dry root rot symptoms were collected for the isolation of pathogen. A fungus was constantly isolated from the infected root tissues and sub cultured on PDA slants. A mycelium of the fungus is ashy-grey in color, septate and branched. It produces large number of small, minute, micro sclerotia. These micro sclerotia are spherical to irregular in shape, black coloured, with mycelial attachment, based on the morphological character, the fungus was identified as *Macrophomina phaseolina* (Fig. 1).

Macrophomina Phaseolina has wide host range and infects pulses, oilseeds and millets and other medicinal plant. Saravanakumar and Samiappan (2006) isolated *Macrophomina phaseolina* from the dry root disease infected Groundnut plants.

Isolation of *Pseudomonas fluorescens* from rhizosphere soil

Pseudomonas is a potential biological control agent against several soil borne pathogens. These beneficial bacteria harbour in the

rhizosphere soil of different crops. *Pseudomonas fluorescens* was successfully isolated from the rhizosphere soil on king's B medium by several authors' including Basha *et al.*, (2014).

In the present study ten isolates of *P. fluorescens* well isolated on king's B medium from rhizosphere soil of different crops grown in the agricultural college and research Institute, Killikulam. Based on the morphological and biochemical characteristics, the bacteria were identified as and named as *Pseudomonas fluorescens* Pfkkm 1 to 10. These bacterial isolates were sub cultured on king's B slants and stored at 5°C for further studies (Table 1 and Fig. 2).

In vitro* screening of *Pseudomonas fluorescens* against *Macrophomina phaseolina

Ten *Pseudomonas fluorescens* isolates were screened for their effectiveness in reducing the mycelial growth of dry root rot pathogens *M. phaseolina* by dual culture method among the ten isolates, seven isolates reduced the mycelial growth of *M. phaseolina* of each

Pfkkm7 of recorded lowest mycelial growth of 10 mm width 88.5% reduction over control. This isolate also recorded max inhibition zone of 51mm. the mycelia growth of 43mm and inhibition zone of 21.33mm. the other isolates like Pfkkm3, Pfkkm4 and Pfkkm10 reduced the mycelial growth but not to the lesser extent on compared to Pfkkm7 and Pfkkm9. the isolates Pfkkm1, Pfkkm2, Pfkkm5, Pfkkm6, did not reduce the mycelia growth of *Macrophomina phaseolina*, (Table 2).

Govindappa *et al.*, (2009) screened 38 *Pseudomonas fluorescens* isolate against *M. phaseolina*. Among these 13 isolates were capable of checking the mycelia growth of *M. phaseolina*.

Development of talc based formulation of *Pseudomonas fluorescens*

Talc based formulation of Pfkkm7 and 9 are prepared by following standard procedure given by Vidhyasekaran and Muthamilan (1995) and these formulations were packed in white polythene bag and stored for further studies.

Table.1 Isolation of *Pseudomonas fluorescens* isolates from rhizosphere soils

S. No	Bacterial Culture	Crop
1	Pfkkm1	Cotton
2	Pfkkm2	Green Gram
3	Pfkkm3	Seasame
4	Pfkkm4	Horse Gram
5	Pfkkm5	Cowpea
6	Pfkkm6	Thenai
7	Pfkkm7	Red Gram
8	Pfkkm8	Sorghum
9	Pfkkm9	Black Gram
10	Pfkkm10	Maize

Table.2 Effect of *Pseudomonas fluorescens* isolates on the mycelia growth of *Macrophomina phaseolina*

S. No	<i>P.fluorescens</i> isolates	Mycelial growth* (mm)	Mycelial growth* (% reduction over control)	Inhibition Zone* (mm)
1	Pfkkm1	87.3 ^e	0.34 ^e	0.47 ^{cd}
2	Pfkkm2	87.6 ^e	0.00 ^e	0.5 ^{cd}
3	Pfkkm3	62.0 ^d	29.20 ^d	3.0 ^{cd}
4	Pfkkm4	86.0 ^e	1.82 ^e	0.43 ^{cd}
5	Pfkkm5	87.6 ^e	0.00 ^e	0.37 ^d
6	Pfkkm6	87.6 ^e	0.00 ^e	0.5 ^{cd}
7	Pfkkm7	10.0 ^a	88.50 ^a	51.0 ^a
8	Pfkkm8	52.0 ^c	40.60 ^c	4.33 ^c
9	Pfkkm9	43.0 ^b	50.91 ^b	21.33 ^b
10	Pfkkm10	86.6 ^e	1.10 ^e	0.57 ^{cd}
11	Control	87.6 ^e	-	-

Table.3 Effect of talc formulation of *Pseudomonas fluorescens* against root rot disease under pot culture experiment

S. No	Treatment	Dosage	Dry root rot incidence (%)*	% reduction over control
1	<i>P. fluorescens</i> Pfkkm 7	10g/kg	24.0 ^b	75.5 ^b
2	<i>P. fluorescens</i> Pfkkm 9	10g/kg	40.0 ^c	59.0 ^c
3	Carbendazim	0.1%	18.0 ^a	81.6 ^a
4	Control	-	98.0 ^d	-

Mean of five replications

In a column, mean followed by a common letter (s) are not significantly different at 5% level by DMRT



Half Plate Technique



Pure culture

Figure 1. Culture of *Macrophomina phaseolina*



Fig 2.Culture of *Pseudomonas fluorescens* on King's slants



T1(Pf1km7) T2 (Pf1km9) T3(Carbendazim) T4(control)

Fig 3. Effect of *Pseudomonas fluorescens* against dry root rot under pot culture condition

Vidhyasekaran and Muthamilan (1995) developed talc formulation of *Pseudomonas fluorescens* isolates Pf1 with the population of 1.3×10^7 cfu/g on 240 days of storage.

Nandakumar *et al.*, (2001) developed talc based formulation *Pseudomonas fluorescens* isolates Pf1 and Pf7.

Effect of *Pseudomonas fluorescens* against dry root rot disease of black gram under pot culture experiment

The talc based formulation of *Pseudomonas fluorescens* isolates Pfkkm7 and Pfkkm9 was evaluated against the dry root rot disease of black gram under pot culture experiment and the result are presented in Table 3. The standard chemical carbendazim was used as check. Among the three treatments the chemical check carbendazim reduced the dry root rot incidence and recorded lesser disease incidence Of 18%.among the two *Pseudomonas fluorescens* isolates Pfkkm7 recorded lesser disease incidence of 24%.

Pseudomonas fluorescens isolated Pfkkm9 also reduced the root rot incidence and recorded 40%.the inoculated control recorded the maximum disease incidence of 98%.the variation in the effectiveness of two isolates might be due to difference in the antibiotic production, HCN production, siderophore production etc. (Fig. 3).

Nandakumar *et al.*, (2000) screened two talc based formulation namely Pf1 and Fp7 against sheath blight pathogen *Rhizoctonia solani* under pot culture experiment.

Saravanakumar and Samiyappan (2006) screened talc based formulation of *Pseudomonas fluorescens* and formed that TDK1 were effective in reducing the dry root rot disease caused by *Macrophomina phaseolina*.

In the present studies also soil application of *Pseudomonas fluorescens* Pfkkm 7 effectively reduced the dry root rot of black gram caused by *Macrophomina phaseolina*.

Environmental and consumer concerns have focused interest on the development of biological control agents as an alternative, environmentally-friendly strategy for the protection of agricultural and horticultural crops against soil borne pathogens. The soil application of *Pseudomonas fluorescens* isolates Pfkkm7 at the rate of 10g/kg of soil effectively reduced the dry root of black gram (75.5%).

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How to cite this article:

Pothiraj, G., A. Kamalakkannan, V. Amirthalingam and Balamurugan, A. 2018. Screening of *Pseudomonas fluorescens* against Dry Root Rot Pathogen *Macrophomina phaseolina* in Black Gram. *Int.J.Curr.Microbiol.App.Sci*. 7(09): 3300-3307.
doi: <https://doi.org/10.20546/ijcmas.2018.709.409>